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(54) Title: PREVENTION OF GELATION OF POLYHYDROXYALKANOATE SOLUTIONS USING SHEAR

(57) Abstract: The present disclosure is of a method of extracting polyhydroxyalkanoate (PHA) from a solution comprising a solvent and the PHA, involving: (i) providing the solution at a first temperature; (ii) cooling the solution to a second temperature, the second temperature effective to precipitate PHA, and concurrently shearing the solution, to yield a precipitated PHA substantially free of gelation; and (iii) recovering the precipitated PHA. In a related way of practicing the method, the solution can be provided by: (a) providing biomass having intracellular PHA; and (b) dissolving the PHA using a solvent and a first temperature effective for dissolving the PHA.

PREVENTION OF GELATION OF POLYHYDROXYALKANOATE SOLUTIONS USING SHEAR

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a process for the extraction and recovery of polyhydroxy-alkanoate (PHA) from biomass.

2. Description of Related Art

There has been considerable interest in recent years in the use of biodegradable polymers to address concerns over plastic waste accumulation. The potential worldwide market for biodegradable polymers is enormous. Some of the markets and applications most amenable to the use of such biopolymers involve those having single, short use applications, including packaging, personal hygiene, garbage bags, and others. These applications, although poorly suited for recycling, are ideally suited for biodegradation through composting.

PHA biopolymers are thermoplastic polyesters produced by numerous microorganisms in response to nutrient limitation. The commercial potential for PHA spans many industries, and is derived primarily from certain advantageous properties which distinguish PHA polymers from petrochemical-derived polymers, namely excellent biodegradability and natural renewability. The success of PHA as a viable alternative to petrochemical-derived polymers, however, will depend upon the design and implementation of efficient and selective means of PHA production and recovery.

An improved understanding of the biology of PHA biosynthetic pathways has allowed for the use of microbial organisms, both natural and recombinant, and more recently plant cells, to produce significant quantities of PHA. Although such approaches have identified promising routes to PHA production, there remain obstacles to efficient and cost-effective PHA recovery from source materials at a useful level of quality and purity.

Alderson, British Patent 2,120,671, describes separation of poly-3-hydroxybutyrate (PHB) from a solution by allowing the solution to gel and then shearing the gel to remove expressed solvent from the mass of discrete gel particles. Solvent remaining in the gel particles is removed by evaporation while stirring the particles.

Haenggi, NATO ASI Ser. E (1990), 186 (Novel Biodegrad. Microb. Polym.), 65-70, describes separation of PHB from biomass by extracting it into an organic solvent, such as chloroform. Dissolved PHB is separated from the aqueous phase and cell debris, and then precipitated from the organic solvent.

Berezovskii et al., Russian Patent 2,051,928, describes mixing a solution of a thermoplastic (e.g. polycarbonate or polysulfone) in a hydrophobic solvent with water, under heating and shear. Water is used as a precipitating agent in order to generate a gel of the polymer with about 5-50 wt% of residual solvent.

Bendix et al., EP 283925, describes purifying polymers dissolved in a solvent by bringing the solution into contact with a precipitating agent under shear. The purpose of the shear is to divide the precipitating polymer into small particles.

SUMMARY OF THE INVENTION

Generally, the present invention relates to a method of extracting PHA from a solution. The method involves cooling the solution under shear. One feature of the method is that the cooling PHA solution does not undergo gelation, but instead forms a flowable slurry of small PHA particles. Such a slurry is readily further processed, without the added expense and time required to prepare a gelled PHA solution for further processing.

In one embodiment, the present invention relates to a method of extracting polyhydroxyalkanoate (PHA) from a solution comprising a solvent and the PHA. The method involves:

providing the solution at a first temperature;

cooling the solution to a second temperature, wherein the second temperature is effective to precipitate PHA, and concurrently shearing the solution, to yield a precipitated PHA substantially free of gelation; and

recovering the precipitated PHA.

In another embodiment, the present invention relates to a method of extracting PHA from a solution of PHA and biomass, involving:

providing biomass having intracellular PHA;

dissolving the PHA using a solvent and a first temperature effective for dissolving the PHA, the resulting solution of PHA having conditions of PHA concentration, solvent, and temperature effective upon cooling to produce a gel;

cooling the solution to a second temperature effective for precipitation of the PHA and concurrently shearing the solution, to yield a precipitated PHA substantially free of gelation; and

recovering the precipitated PHA.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The embodiments disclosed herein relate to novel methods for the recovery of PHA polymer from a solution comprising a solvent and the PHA. In one group of embodiments, the solution comprises a solvent and a PHA extracted from biomass materials, wherein the biomass materials are derived from PHA-producing plants or PHA-producing microorganisms.

In one embodiment, the present invention is directed to a method of extracting polyhydroxyalkanoate (PHA) from a solution comprising a solvent and the PHA, comprising:

providing the solution at a first temperature;

cooling the solution to a second temperature, wherein the second temperature is effective to precipitate PHA, and concurrently shearing the solution, to yield a precipitated PHA substantially free of gelation; and

recovering the precipitated PHA.

The PHA can be any PHA known in the art or subsequently produced. The methods of the present invention are applicable to the recovery of all types of PHA polymer. PHA is a polymer made from repeating units having the following general structure:

$$H\!-\!\left[\text{O-}\overset{\overset{\displaystyle R_1}{|}}{\text{CH}}\text{-}(\text{CH}_2)_p\text{-}\overset{\overset{\displaystyle O}{\|}}{\text{C}}\right]_n\!-\!H$$

wherein R_1 is preferably an H, alkyl, or alkenyl; p is 0, 1, 2, or 3; and n is an integer. PHA can consist entirely of a single monomeric repeating unit, in which case it is referred to as a homopolymer. For example, poly-3-hydroxybutyrate (PHB) homopolymer has repeating monomeric units wherein $R_1=C_1$ alkyl, and p=1. For another example, poly-4-hydroxybutyrate (P4HB) homopolymer has repeating monomeric units wherein $R_1=H$ and p=2. Polyhydroxyvalerate homopolymer has repeating monomeric units wherein $R_1=C_1$ alkyl, and p=1. Copolymers, in contrast, contain two different types of monomeric units. For example, one unit could contain $R_1=C_1$ alkyl and p=1, and the other repeating unit could contain $R_1=C_2$ alkyl, and p=2. When three different types of repeating units are present the polymer is referred to as a terpolymer. The methods disclosed herein may also be applicable to the recovery of PHA which

has been modified to provide improved or beneficial properties, for example by modification to contain hydroxy-terminated end groups.

The polymers described above, whether homo-, co-, or terpolymers, can be of any desirable molecular weight Mw. ("Molecular weight" or "Mw," unless stated otherwise, shall refer to the weight average molecular weight determinable by methods well known to one of ordinary skill in the art). Preferably, the Mw is greater than about 20 kD. More preferably, the Mw is in the range of from about 60 kD to about 500 kD. Most preferably, the Mw is in the range of from about 100 kD to about 400 kDa.

The solvent can be any solvent in which the PHA dissolves. Preferably, the solvent selected is one in which the PHA is highly soluble. It should be noted that PHA composition and morphology (presence or absence of crystallinity, and if present, the percent crystallinity) are determinants of polymer solubility characteristics. Therefore, composition and morphology of the specific PHA being recovered are important considerations when selecting suitable solvents for use in the present invention. PHBH (C_4/C_6) copolymer is typically more soluble than PHBV (C_4/C_5) copolymer, which is typically more soluble than PHB (C_4) homopolymer. In this regard, a C_4 alcohol, for example, may be a suitable solvent for a particular PHA, but may not be a suitable solvent for a different PHA, depending upon polymer composition.

Solvents useful in the present invention can be selected from the group consisting of cyclic and acyclic (linear and branched) R'-OH alcohols where R'= C_4-C_{10}, cyclic and acyclic R''-COOR''' esters where R''=H or C_1-C_6 and R'''=C_1-C_6, cyclic and acyclic R''-COOR''' esters where R''=H or C_1-C_6 and R'''=C_1-C_6 and wherein at least one oxygen is substituted for at least one carbon in R'' or R''', cyclic and acyclic R^1-CON-$(R^2)_2$ amides where R^1 = H or C_1-C_6 and R^2 = C_1-C_6, and cyclic and acyclic R^3-CO-R^4 ketones where R^3=C_1-C_6 and R^4=C_1-C_6. Preferred solvents for use in the methods of the present invention include dimethyl formamide, dimethyl acetamide, methyl ethyl ketone, methyl amyl ketone, methyl isobutyl ketone, butyl acetate, ethyl butyrate, propyl butyrate, butyl butyrate, methyl propionate, propyl propionate, butyl propionate, pentyl propionate, methyl valerate, ethyl valerate, 2-butanol, 2-methyl-1-propanol, 1-pentanol, 3-pentanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-hexanol, 1-heptanol, cyclohexanol, 2-ethyl hexanol, 2-methoxy ethyl acetate and tetrahydrofurfuryl

acetate. Other preferred solvents include ethyl lactate, tetrahydrofurfuryl alcohol, gamma-butyrolactone, 1,2-dimethoxybenzene, and 1,3-dimethoxybenzene.

A group of solvents that is generally preferred for many PHAs includes amyl alcohol, hexyl alcohol, ethylene glycol diacetate, 2-heptanone, butyl butyrate, 2-methyl 1-butanol, other alcohols, esters, and dioxanes. Amyl alcohol is especially preferred.

The PHA concentration in the PHA-enriched solvent is typically between about 1% and about 40% w/v.

The temperature of the solution before cooling (herein the "first temperature") can be any temperature in which the PHA is substantially soluble that is below the boiling point of the solvent and the melting point of the PHA. Typically, the solvents will have boiling points above 75°C at atmospheric pressure. More particularly, boiling points will be in the range of 100-180°C. Melting points of PHAs are typically in the range of 130-180°C. The pressure of the solution before cooling can be any pressure useful in PHA processing, and most readily will be 1 atm.

It is desirable that the rate and extent of shear during cooling be such to prevent gelation, i.e. to produce PHA in the form of discrete particles of small size (about 1 mm in diameter or less). The Examples below describe the use of magnetic stirrers or impellers to provide stirring of at least about 100 rpm, preferably about 150 rpm to about 1000 rpm, for benchtop work (flask volume 500 mL). Factors that will determine the rpm required for a stirrer or impeller to generate sufficient shear in an industrial-scale embodiment include: the volume of the vessel, the concentration of PHA in the solution, the size of the paddle or paddles of the stirrer or impeller, and the temperature to which the solution is to be cooled. Alternatively, shear can be generated by sonication or other known methods. The process conditions required to generate sufficient shear will be readily determined, without undue experimentation, by one of ordinary skill in the art.

Cooling the solution can be performed by any known method. Preferably, cooling is performed by circulating air or water partially or completely around the vessel in which the solution is to be cooled. The rate of cooling can be any rate possible using air- or water-cooling. The temperature to which the solution is cooled (the "second temperature") can be from about 30°C to about 90°C, preferably from about 50°C to about 80°C, and in any event will be below the first temperature. The lowest temperature to which the solution can be cooled without gelation will depend, as one of skill in the art will recognize, on the PHA used, the solvent used,

and the rate of shear. For example, use of amyl alcohol as the solvent may result in gelation below about 50°C, whereas use of ethylene glycol diacetate may allow cooling to about 30°C. The minimum temperature to which the solution can be cooled will be readily determined, without undue experimentation, by one of ordinary skill in the art. The second temperature can be any temperature which is acceptable for further processing, as described below.

After the solution has been cooled to the desired second temperature, PHA will be present as a slurry of discrete particles in the solution. PHA can be separated from the solvent by any method know in the art, for example, by filtration or centrifugation. The separated PHA can then undergo any other processing or manufacturing steps as known to one of ordinary skill in the art. After separation of the PHA, the solvent may be reused, either directly, by dilution into fresh solvent, or by distillation to separate the used solvent from any impurities therein. Other uses for the solvent may be apparent to one of ordinary skill in the art.

One advantage of this approach relates to the ease with which PHA can be recovered. Upon cooling of the PHA-enriched solvent mixture of the present invention under shear, a precipitate forms which is easily removed from the solvent. In contrast, other solvent systems used for PHA extraction which involve subsequent recovery of PHA by temperature reduction typically result in a PHA-enriched solvent which forms a stable gel upon cooling. Additional steps are then required in order to separate PHA polymer from the gel. For example, the gel can be compressed into flakes, and remaining solvent present in the flakes can be removed by heat evaporation.

Other polymer recovery approaches require the use of precipitating agents to precipitate PHA from a PHA-enriched solvent. As defined herein, a "precipitating agent" refers to a second solvent or second solution in which the PHA is substantially (at least 50% w/v) less soluble than it is in the solvent recited in the above method.

The present invention advantageously provides methods for the recovery of PHA polymer without undesirable gel formation and without the requirement for precipitating agents by cooling the PHA-enriched solvent under shear.

In another embodiment, the present invention relates to a method of extracting PHA from a solution of PHA and biomass, comprising:

providing biomass having intracellular PHA;

dissolving the PHA using a solvent and a first temperature effective for dissolving the PHA, the resulting solution of PHA having conditions of PHA concentration, solvent, and temperature effective upon cooling to produce a gel;

cooling the solution to a second temperature effective for precipitation of the PHA and concurrently shearing the solution, to yield a precipitated PHA substantially free of gelation; and

recovering the precipitated PHA.

Usable solvents, PHAs, PHA concentrations, temperatures at which the PHA is dissolved, temperatures to which the PHA is cooled, the technique and rate of cooling, and the extent of shear, are as described above.

The biomass can be any biomass containing intracellular PHA. Preferably, the biomass is microbiological or plant material. Microbiological biomass can be derived from any unicellular organism that produces PHA, such as bacteria or yeast. The production of PHA by the unicellular organism can be a characteristic of the organism as found in nature, a characteristic engineered in the organism by genetic engineering techniques, or both. An exemplary unicellular organism that produces PHA is *Alcaligenes eutrophus*. Others are known to one of ordinary skill in the art.

If the biomass is plant material, the methods are applicable, for example, to PHA recovery from plants belonging to taxa having chlorophyll a and optionally one or more other chlorophylls in their photosynthetic apparatus. The plants can be monocots or dicots. Suitable plant source materials from which PHA can be recovered include roots, stems, leaves, flowers, fruits, or seeds.

The embodiments of the present invention are particularly useful for PHA recovery from oil-bearing seeds. Oilseed crops, such as canola, rapeseed, soybean, safflower, and sunflower, can be genetically engineered with the result of PHA being biosynthetically produced in the seeds of the crops. In order to recover PHA polymer from the seeds, it is necessary to separate the polymer from the vegetable oil and oilseed meal also present. The seeds are typically processed by conventional methods. For example, they can be crushed, dehulled, oil-extracted, or protein extracted, in any order, prior to PHA extraction. The oilseed meal which is separated from the PHA-enriched solvent may be further processed and used as animal feed or an additive in animal feed.

Prior to the dissolving step, the biomass can be pretreated. "Pretreatment" herein means removal or degradation of non-PHA cellular material, such as proteins, glycoproteins,

oligosaccharides, other carbohydrates, and nucleic acids, in order to facilitate dissolving of PHA (by which is meant increasing the yield, the purity, or both of the PHA) in the solvent. Techniques of pretreatment that are known to be effective include grinding, chemical digestion (e.g. by surfactants or peroxide), or enzymatic digestion (e.g. by proteinases or lysozyme).

EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1: 4.0 g of polyhydroxybutyrate-co-valerate (PHBV (8% HV)) was added to 100 cc of amyl alcohol in a 140 ml beaker containing a magnetic stirrer. The mixture was heated to 112°C on a hot plate while being stirred to obtain a clear solution of PHA in amyl alcohol. At that point, the heater was turned off and the solution was allowed to air-cool while being stirred by the magnetic stirrer (and occasionally manually with a spatula). Upon cooling to 57°C, the solution had not gelled and the entire slurry was fluid and could be filtered easily.

Example 2: 6.03 g of PHBV (8% HV) was added to 150 ml amyl alcohol, which was preheated to 108°C in a 500 ml reaction flask equipped with a Lightnin R500® (Lightnin Mixing Equipment; Rochester, NY) impeller. The mixture was heated to 125°C in a hot oil bath while being stirred at 250 rpm to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred at 250 rpm. Upon cooling to 61°C, the solution did not gel and the entire slurry of very fine polymer particles was fluid and could be filtered very easily.

Example 3: 9.0283 g of PHBV (8% HV) was added to 150 ml amyl alcohol, preheated to 112°C, in a 500 ml reaction flask equipped with a Lightnin R500 impeller. The mixture was heated to 126°C in a hot oil bath while being stirred at 200 rpm to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred at 250 rpm. Significant polymer precipitation was observed around 84°C. Upon cooling to 58°C, the solution did not gel and the entire slurry of very fine and discrete polymer particles in amyl alcohol was fluid and could be filtered very easily.

Comparative Example 3a: The solution produced in Example 3 was reheated to 123°C in the hot oil bath to obtain a clear solution and the same cooling procedure repeated, except that the solution was stirred at 100 rpm. Upon cooling to 55°C, the solution gelled and some chunks of broken polymer gel were observed.

Example 4: 12.02 g of PHBV (8% HV) was added to 150 ml amyl alcohol, preheated to 112°C in a 500 ml reaction flask equipped with a Lightnin R500 impeller. The mixture was heated to 123°C in a hot oil bath while being stirred at 250 rpm to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred at 300 rpm. Upon cooling to 47°C, the polymer solution gelled.

Example 5: 8.00 g of poly-3-hydroxybutyrate-co-4-hydroxybutyrate (P(3HB4HB), (11.6% 4HB)) was added to 200 ml amyl alcohol, preheated to 113°C in a 500 ml reaction flask equipped with a Lightnin R500 impeller. The mixture was heated to 127°C in a hot oil bath while being stirred at 100 rpm to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred at 300 rpm. Upon cooling to 57°C, the solution did not gel and the entire slurry of fine and discrete polymer particles was fluid and could be filtered very easily.

Comparative Example 5a: The solution prepared in Example 5 was reheated to 115°C in the hot oil bath to obtain a clear solution and the same cooling procedure repeated, except that the solution was stirred at 80 rpm. Upon cooling to room temperature, the solution gelled and some chunks of broken polymer gel were observed.

Example 6: 0.9328 g of poly-hydroxyl-terminated-hydroxybutyrate (PHB-OH, (90% hydroxyl-terminated)) was added to 30 cc of amyl alcohol in a 100 ml round bottle containing a magnetic stirrer. The mixture was heated to 123°C in a hot oil bath while being stirred to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred by the magnetic stirrer. The polymer solution remained flowable until it geled. Upon cooling to room temperature (e.g. ~22°C), the solution gelled.

Example 7: 8.0048 g of PHBV (8% HV) was added to 200 ml hexyl alcohol preheated to 125°C in a 500 ml reaction flask equipped with a Lightnin R500 impeller. The polymer was completely dissolved at this temperature and a clear solution of PHBV (8% HV) in hexyl alcohol was obtained. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred at 300 rpm. Upon cooling to 59°C, the solution did not gel and the entire slurry of fine and discrete polymer particles was fluid and could be filtered very easily.

Comparative Example 7a: The solution produced in Example 7 was reheated to 119°C in the hot oil bath to obtain a clear solution and the same cooling procedure repeated, except that the solution was not stirred. Upon cooling to room temperature, the solution gelled.

Example 8: 6.0014 g of PHBV (8% HV) was added to 150 ml ethylene glycol diacetate, preheated to 108°C in a 500 ml reaction flask equipped with a Lightnin R500 impeller. The mixture was heated to 124°C in a hot oil bath while being stirred at 300 rpm to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred at 300 rpm. Upon cooling to 32°C, the polymer solution did not gel, but discrete polymer precipitate particles were not visually observed.

Example 9: 4.5001 g of PHBV (8% HV) was added to 150 ml 2-heptanone preheated to 123°C in a 500 ml reaction flask equipped with a Lightnin R500 impeller. The polymer was completely dissolved at this temperature and a clear solution of PHBV (8% HV) in 2-heptanone was obtained. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred at 300 rpm. Upon cooling to 51°C, the polymer solution did not gel and polymer could be easily recovered by filtration although discrete polymer precipitate particles were not visually observed.

Comparative Example 10: 0.2995 g of PHBV (8% HV) was added to 10 ml 2-heptanone in a test tube. The mixture was heated in a hot oil bath to 128°C while being stirred by a magnetic stirring bar to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool without agitation. The solution was then cooled until it gelled. There was negligible amount of clear solvent observed in this polymer gel after being centrifuged.

Example 11: 3.0059 g of PHBV (8% HV) was added to 150 ml butyl butyrate preheated to 115°C in a 500 ml reaction flask equipped with a Lightnin R500 impeller. The mixture was heated in a hot oil bath to 132°C while being stirred at 200 rpm to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool while being stirred at 300 rpm. Significant polymer precipitation was observed at 80°C. Upon cooling to 42°C, the polymer solution did not gel and polymer could be easily recovered by filtration, although discrete polymer precipitate particles were not visually observed. A portion of the solution was centrifuged and some clear solution on top of polymer precipitate was observed.

Comparative Example 12: 0.3000 g of PHBV (8% HV) was added to 15 ml butyl butyrate in a test tube. The mixture was heated in a hot oil bath to 132°C while being stirred by

a magnetic stirring bar to obtain a clear solution. At that point, the hot oil bath was removed and the solution was allowed to air-cool without agitation. Upon cooling, the polymer solution gelled. There was negligible amount of clear solvent observed in this polymer gel after being centrifuged.

Example 13: A mixture of corn stover / PHBV (8% HV) was prepared by blending 30.0 g dry ground corn stover with 5.3 g PHBV. This pre-blended biomass was contacted with 178 ml amyl alcohol at 127°C for 90 minutes in a 500 ml reaction flask equipped with a Teflon single-blade impeller. After the polymer extraction / dissolution, the biomass-PHA solution slurry was filtered using a pressure filter (D porosity, 10 - 20 mm). The pressure filter was maintained at 122°C to avoid polymer precipitation and loss in the biomass residue. The filtration was easy to perform with 2 - 3 psi N_2 pressure differential. A dark brown colored polymer solution was collected in a 250 ml three-neck round bottle which was maintained at 122°C in a hot oil bath. Then the hot oil bath was removed and the polymer solution was allowed to air cool with the agitation of a star-head magnetic stirrer in the round bottle. Upon cooling to 80°C, significant polymer precipitation occurred and the polymer solution became slurry. The polymers was recovered by filtration using a pressure filter (D porosity, 10 - 20 mm) maintained at 80°C, using a 5 - 7 psi N_2 pressure differential. The polymer cake was then washed using fresh amyl alcohol at 80°C in the filter. After drying the wet cake, 2.16 g dry polymer was obtained (41%), which had a white color with a little yellow tint.

All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

CLAIMS:

1. A method of extracting polyhydroxyalkanoate (PHA) from a solution comprising a solvent and the PHA, comprising:

providing the solution at a first temperature;

cooling the solution to a second temperature, wherein the second temperature is effective to precipitate PHA, and concurrently shearing the solution, to yield a precipitated PHA substantially free of gelation; and

recovering the precipitated PHA.

2. The method of claim 1, wherein the solvent is selected from amyl alcohol, 2-methyl 1-butanol, hexyl alcohol, ethylene glycol diacetate, 2-heptanone, butyl butyrate, other alcohols, esters, and dioxanes.

3. The method of claim 2, wherein the solvent is amyl alcohol.

4. The method of claim 1, wherein the PHA concentration is between about 1% and about 40% w/v, and the first temperature is at least 75°C.

5. The method of claim 4, wherein the first temperature is from about 100°C to about 180°C.

6. The method of claim 1, wherein the cooling is to a temperature of from about 30°C to about 90°C.

7. The method of claim 6, wherein the cooling is to a temperature of from about 50°C to about 80°C.

8. A method of extracting PHA from a solution of PHA and biomass, comprising:

providing biomass having intracellular PHA;

dissolving the PHA using a solvent and a first temperature effective for dissolving the PHA, the resulting solution of PHA having conditions of PHA concentration, solvent, and temperature effective upon cooling to produce a gel;

cooling the solution to a second temperature effective for precipitation of the PHA and concurrently shearing the solution, to yield a precipitated PHA substantially free of gelation; and

recovering the precipitated PHA.

9. The method of claim 8, wherein the biomass is microbiological or plant material.

10. The method of claim 8, wherein the solvent is selected from amyl alcohol, 2-methyl 1-butanol, hexyl alcohol, ethylene glycol diacetate, 2-heptanone, butyl butyrate, other alcohols, esters, and dioxanes.

11. The method of claim 10, wherein the solvent is amyl alcohol.

12. The method of claim 8, wherein the PHA concentration is between about 1% and about 40% w/v, and the first temperature is at least 75°C.

13. The method of claim 12, wherein the first temperature is from about 100°C to about 180°C.

14. The method of claim 8, wherein the cooling is to a temperature of from about 30°C to about 90°C.

15. The method of claim 14, wherein the cooling is to a temperature of from about 50°C to about 80°C.

16. The method of claim 8, further comprising pretreating the biomass between the providing and the dissolving steps.

17. The method of claim 16, wherein the pretreating is performed by grinding, chemical digestion, or enzymatic digestion.